



## Evaluation of *In-vitro* Anthelmintic Activity of *Catharanthus roseus* Extract

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### ABSTRACT

Helminthes infections are chronic illnesses in human beings and in cattle. *Pherithema posthuma* a helminthes is commonly known as earth-worms. Although the use of alternate drugs has been as a remedial measure against the resistant strains of helminth parasites, and as a means of reducing the cost of controlling helminthic diseases. *Catharanthus roseus* is a medicinally valuable plant and possess various pharmacological properties. *Catharanthus roseus* has been traditionally used as an anthelmintic agent. To justify the ethnomedical claims, the anthelmintic property of *Catharanthus roseus* was evaluated using *Pherithema posthuma* as an experimental model. Piperazine citrate was used as the standard reference. Among the various concentrations tested, ethanol extract at 200 mg/ml showed efficient paralysis effect (6.67 min) than other treated groups, whereas ethanol extract 250 mg/ml showed significant anthelmintic activity with death time of 46.33 min. Standard drug at 50 mg/ml showed paralysis at 31.33 min and death time was 40.67 min. This investigation revealed that ethanol extract of *Catharanthus roseus* showed significant anthelmintic activity against *Pheretima posthuma*. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *Catharanthus roseus* as an anthelmintic plant.

**Keywords:** Apocynaceae, *Catharanthus roseus*, anthelmintic activity, *Pheretima posthuma*, ethanol extract.

### INTRODUCTION

Helminthes infections are commonly found in community and being recognized as cause of much acute as well as chronic illness among the various human beings as well as cattle's. More than half of the population of the world suffers from various types of infection and majority of cattle's suffers from worm infections. [1] However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelmintics [2] has led to development of resistance and hence a reduction in the usefulness of available anthelmintics. [3] Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites and as a means of reducing the cost of controlling helminthic diseases. [4-7] The plants are known to provide a rich source of botanical anthelmintics. [8-10] A number of medicinal plants have been used to treat parasitic infections in man and

animals. [11-12]

*Catharanthus roseus* (Madagascar periwinkle) is a species of *Catharanthus* native and endemic to Madagascar. Synonyms include *Vinca rosea*, *Ammocallis rosea*, and *Lochnera rosea*; other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and "Old-maid". In the wild, it is an endangered plant; the main cause of decline is habitat destruction by slash and burn agriculture. It is also however widely cultivated and is naturalized in subtropical and tropical areas of the world. [13] It is an evergreen sub-shrub or herbaceous plant growing to 1 m tall with white to dark pink flowers.

Alkaloids isolated from *C. roseus* are hypotensive, sedative and have tranquilising properties and are anti cancerous. Traditionally it is used for relieving muscle pain, depression of central nervous system and wasps stings. It is used in case of nose bleed, bleeding gums, mouth ulcers and sore throats. It is also used internally for loss of memory, hypertension, cystitis, gastritis and enteritis, diarrhoea and raised blood sugar levels. [14] Its application ranges from prevention of cancer, cancer treatment, anti-diabetic, stomachic, reduces high blood pressure, externally against nose bleeding, sore throat and mouth ulcer. [15] *C. roseus* has been traditionally

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used as an anthelmintic agent. However, anthelmintic activity of *C. roseus* whole plant extract is not scientifically proved and reported. To justify the traditional claims of *C. roseus*, we made an efficient attempt to assess the anthelmintic activity of *C. roseus*.

## MATERIALS AND METHODS

### Drugs and chemicals

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Ethanol was purchased from Hong, Yang Chemical Corporation, China.

### Plant Resource

*Catharanthus roseus* plant material was collected from Bhadra Wildlife Sanctuary, Western Ghats, India. The plant was authenticated by Prof. V. Krishna, Kuvempu University. Fresh plant material was washed thoroughly in tap water to remove traces of soil and other contaminants. It is then shade dried. Further, the whole plant was chopped finely and was shade dried, powdered mechanically and was subjected to cold extraction using ethanol as the solvent system for about 96 h, after every 24 h fresh ethanol was added and ethanol containing the extract was separated. The ethanol extract was filtered and concentrated in vacuum under reduced pressure and allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of ethanol crude extract for 1 kg of powdered plant material was 45 g.

### Test organism

Indian adult earthworms (*Pheretima posthuma*) collected from the Indo-American Hybrid Seeds, Bangalore. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water, for about two weeks. Before the initiation of experiment the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for the experiment. This organism was selected model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.<sup>[16-17]</sup>

### Extract preparation for experiment

The porously powdered plant material was used for extract preparation. After extraction, the crude extract was stored in dessicator until further use. Ethanol extract and standard drug piperazine citrate were dissolved in 0.5% DMSO in normal saline (v/v) and used for evaluation for anthelmintic activity.

### Anthelmintic activity

The anthelmintic activity of whole plant extracts of *Catharanthus roseus* was evaluated as per the method reported by Dash *et al.*<sup>[18]</sup> Seven groups of animals with three earthworms in each groups, each earthworm were separate released into 20 ml of desired formulation in normal saline, Group I earthworm were released in 20 ml normal saline in a clean petri plate and were maintained as control. Group II, III, IV, V, VI earthworms were released in 50, 100, 150, 200 and 250 mg/ml of ethanol extract in 20 ml of normal saline respectively. Group VII earthworms were released in normal saline containing standard drug piperazine citrate (50 mg/ml). Earthworms were observed; the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total loss of motility with faded body

color.<sup>[19]</sup> The result of anthelmintic activity is depicted in Table 1.

### Statistical analysis

The data of anthelmintic evaluations were expressed as mean  $\pm$  S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at  $P < 0.01$  was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

## RESULTS AND DISCUSSION

*Catharanthus roseus* is a well known medicinal plant and is widely used in folk medicine/ ayurvedic system of medicine. In the present study solvents namely ethanol and water were used sequentially for crude extraction of *Catharanthus roseus* whole plant. To justify the ethnomedical claims of *Catharanthus roseus* we made an efficient attempt in evaluating the anthelmintic property of *Catharanthus roseus*.

**Table 1: *In-vitro* anthelmintic activity of ethanol extract of *Catharanthus roseus* against *Pheretima posthuma***

Test samples	Concentration (mg/ ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Normal Saline)		64.33 $\pm$ 0.88	200.33 $\pm$ 2.60
Ethanol extract of <i>Catharanthus roseus</i>	50	42.33 $\pm$ 1.45**	83.67 $\pm$ 2.16**
	100	32.33 $\pm$ 1.86**	73.00 $\pm$ 1.73**
	150	17.33 $\pm$ 0.88**	68.67 $\pm$ 1.45**
	200	6.67 $\pm$ 1.45**	51.67 $\pm$ 0.88**
250	20.33 $\pm$ 1.33**	46.33 $\pm$ 0.88**	
Piperazine citrate	50	31.33 $\pm$ 1.86**	40.67 $\pm$ 0.88**

Values are the mean  $\pm$  S.E.M. of three earthworms. Symbols represent statistical significance. \*  $P < 0.05$ , \*\*  $P < 0.01$ , ns: not significant as compared to compared to control group

Earthworms belonging to control group showed paralysis time at 64.33 and death time at 200.33 min. Whereas, the ethanol extract at the concentration of 50 mg/ml showed the time of paralysis and death at 42.33 and 83.67 min respectively. For concentration of 100 mg/ml, the paralysis and the death time was found to be 32.33 and 73 min respectively. At the concentration of 150, 200 and 250 mg/ml, time taken to paralysis was 17.33, 6.67 and 20.33 min respectively and death time 68.67, 51.67 and 46.33 min respectively.

Among the various concentrations tested, ethanol extract at 200 mg/ml showed efficient paralysis effect (6.67 min) than other treated groups, whereas ethanol extract 250 mg/ml showed significant anthelmintic activity with death time of 46.33 min (Table 1). Standard drug at 50 mg/ml showed paralysis at 31.33 min and death time was 40.67 min (Figure 1). This investigation revealed that ethanol extract of *Catharanthus roseus* showed significant anthelmintic activity against *Pheretima posthuma*. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *Catharanthus roseus* as an anthelmintic plant.

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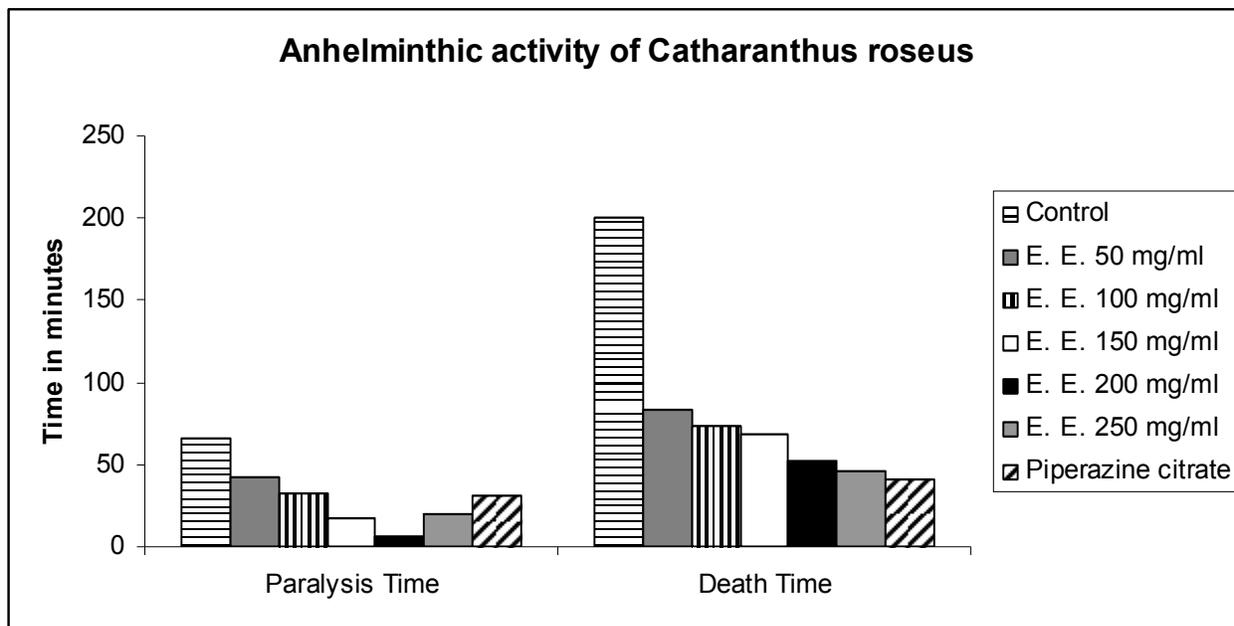


Fig. 1: Graph showing the effects of ethanol extract of *Catharanthus roseus* on *Pheretima posthuma*

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